UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 07 2007

MEMORANDUM

SUBJECT: Review of follow-up resistance monitoring data for a Cry1F tolerant ECB population collected in Hamilton County, Iowa during 2004. Data submitted by Dow AgroSciences for Herculex Bt corn registrations 68467-2 and 29964-3. DP Barcode: 336805. Decision: 373283. MRID#: 466958-01 and 470112-01.

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8/7/07

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8/7/07

Action Requested

BPPD[1] has been asked to review supplemental resistance monitoring information submitted by Dow AgroSciences for a Cry1F-tolerant European corn borer population collected from Hamilton County, Iowa in 2004. The submitted materials (MRID# 466958-01 and 470112-01) include data for the Cry1F toxin in support of the Herculex Bt corn products (EPA Registration Nos. 68467-2 and 29964-3); similar data developed for the Cry1Ab toxin has been submitted and reviewed separately (see BPPD 2007).

Conclusions and Recommendations

1) Dow's submitted report clearly demonstrates that the European corn borer (ECB) population collected in 2004 from Hamilton County, Iowa is resistant to the Cry1F toxin expressed in Herculex corn. The colony meets all of the major criteria for resistance: the trait is heritable

[1] The use of BPPD in this review refers to the BPPD IRM Team consisting of Sharlene Matten and Alan Reynolds

(determined to be a single recessive gene), the trait confers survival to high levels of Cry1F, and ECB with the trait are capable of developing to adults on Cry1F-expressing corn plants. Overall, tested ECB exhibited a resistance to Cry1F levels that exceeded 2,000 to 6,000 times the LC_{50} for susceptible ECB. BPPD notes that this is the first documented case of pest resistance to a high dose Bt crop; though it should also be noted that no documented cases of field failure or resistance have been detected in Hamilton County or elsewhere.

2) BPPD recommends continued sampling of ECB in the Hamilton County region (i.e. Hamilton County and the surrounding counties) during subsequent growing seasons as part of the annual ABSTC monitoring program. Such monitoring will be important to ascertain whether (and at what levels) the Cry1F tolerance trait is still present in the region. Additional monitoring techniques (such as the F_2 screen) should also be considered to determine the frequency of recessive resistance.

3) BPPD recommends that Dow (or ABSTC) continue to maintain and investigate the HC colony. This colony may yield valuable information on Cry1F resistance that may aid the understanding and improvement of IRM plans. As part of this work, any fitness costs of Cry1F tolerance in the Hamilton County colony should be examined to characterize potential field survival and resistance proliferation. Additional monitoring techniques (such as the F_2 screen) should also be considered to determine the frequency of recessive resistance. Further, it is recommended that Dow investigate cross-resistance potential between Cry1F and Cry1Ab since the Hamilton County ECB population exhibited tolerance to both toxins.

4) The slide presentation (MRID# 466958-01) submitted to describe initial testing conducted during 2005 (i.e. tests through the F_8 generation) did not include a description of the methods used in the assays. Rather, abbreviated results tables and figures were presented. BPPD recommends that future reports be properly formatted with full descriptions of the methods, results, and appropriate statistical analyses.

Background

As a primary target pest of Bt corn, European corn borer (ECB) populations have been monitored for susceptibility to Cry1Ab since the 1995 growing season (diagnostic concentration information has been collected since 1999) and to Cry1F since the 2000 season. All of the ECB monitoring assays to date have been conducted by Dr. Blair Siegfried, an entomologist at the University of Nebraska. Monitoring has also been required since 1998 for corn earworm (CEW), southwestern corn borer (SWCB), and fall armyworm (FAW, a target pest of Bt sweet corn) susceptibility to Cry1Ab (Cry1F monitoring started in 2001). All of the Cry1Ab and Cry1F monitoring data from the 2001 through the 2005 growing season has been previously reviewed by the Agency (see BPPD 2004a, b, 2006, 2007).

The 2004 resistance monitoring data described one ECB population, collected from Hamilton County, Iowa, exhibited substantially higher tolerance to both Cry1Ab and Cry1F than the other tested ECB populations. This population had a Cry1Ab LC_{50} of 19.86 ng/cm^2 (compared with 1.74 - 5.24 ng/cm^2 for the other collected populations) and a Cry1F LC_{50} of 45.42 ng/cm^2

(compared with 3.76 - 9.73 ng/cm^2 for the other populations). Additionally, the Hamilton County population also had mortalities of 50.68% on the Cry1Ab diagnostic concentration and 48.35% for the Cry1F diagnostic concentration. The diagnostic concentrations for ECB have been based on the LC_{99} for the insect and the assays typically produce at least 99% mortality in susceptible populations.

BPPD noted in its review of the data (see BPPD 2006) that it was still too early to definitively classify this population as "resistant" to the Cry1Ab and/or Cry1F toxins and that no reports of unexpected ECB damage in the field to Bt corn were received from this region. However, ABSTC was required to implement a series of follow-up tests with the Hamilton County population, as described in the Bt corn resistance monitoring strategy for this population (see ABSTC 2003). The goal of this additional testing was to determine: 1) if the observed effect is heritable; 2) if the increased tolerance can be observed in the field (i.e. on live Bt corn plants); 3) if the effect is due to resistance, the nature of resistance (dominant, recessive); 4) the resistance allele frequency; 5) whether the resistance allele frequency is increasing; 6) the geographic extent of the resistance allele distribution.

Due to proprietary reasons, reports on the Hamilton County follow-up testing for Cry1Ab were submitted separately from Cry1F. The Cry1Ab data (pertaining to Yieldgard products registered by Monsanto and Syngenta) were submitted by ABSTC and reviewed as part of the 2005 monitoring report (see BPPD 2007). Data for Cry1F were subsequently submitted by Dow AgroSciences, the registrant of Herculex corn, and are reviewed in this memorandum. Two reports were submitted by Dow to cover the Cry1F activities for the Hamilton County population. The first (MRID# 466958-01) was a slide presentation that described a progress report from the first set of studies conducted in 2005 (based on a briefing given to BPPD in November, 2005). The second submission (MRID# 470112-01) included a full report of the remaining follow-up studies conducted during 2006.

BPPD's review of the Cry1Ab follow-up testing concluded that bioassays conducted on Cry1Ab tolerant ECB populations from Hamilton County collected in 2004 showed that individuals were incapable of surviving on Cry1Ab-expressing corn plants. ABSTC followed the appropriate procedures for follow-up testing on populations that show tolerance to Bt toxins and no further testing is needed for Cry1Ab. Further, testing of new populations collected from Hamilton Co. in 2005 indicated no reduced susceptibility to Cry1Ab or Cry1F (note: the ABSTC report included data with both toxins tested with the 2005 ECB collections). BPPD also recommended that ABSTC continue to sample ECB in Hamilton County in future growing seasons to detect any additional changes to ECB susceptibility. For the 2005 growing season, ABSTC collected additional ECB populations in Hamilton County along with collections from other corn-growing regions. Susceptibility and diagnostic bioassays showed that unlike 2004, the 2005 Hamilton County collections were not tolerant to either Cry1Ab or Cry1F (see BPPD 2007). Additional collections were to be made in 2006 as well, though data have not been reported at the time of this review.

Follow-up Testing Conducted on the 2004 Hamilton County Colony

Dow's follow-up testing on the Hamilton County populations focused on four major objectives: 1) the level and heritability of the potential resistance; 2) survival on Bt (Cry1F) expressing plants; 3) the genetics of the potential resistance; and 4) the frequency of resistance in field populations from Hamilton County. The bioassays involved in these objectives were conducted by Dr. Blair Siegfried's laboratory at the University of Nebraska. Dr. Siegfried has conducted the ECB monitoring for Bt corn since 1995.

Initial testing of the Hamilton County ECB population (that was done as part of the 2004 monitoring work) was conducted on the F_3 and F_4 generation. Bioassay results from these individuals revealed the significantly higher Cry1F tolerance that initiated the follow-up testing described below. Since this Cry1F tolerance was observed, the colony has been maintained for additional generations using the survivors from the F_3/F_4 diagnostic concentration assays. The colony was reared on artificial non-Bt diet (no Cry1F challenges other than those described below have been part of the colony rearing) and subsequent generations (F_7 through F_{18}) were used in the bioassays to investigate potential resistance.

1) Level and Heritability of Resistance

The first step in Dow's investigation of the Hamilton County ECB population was to determine if the elevated Cry1F tolerance was a heritable trait. Heritability is a key component of resistance development; without the ability to pass a resistance trait on to subsequent generations the effect will not be maintained in the population.

Heritability testing was first conducted on F_7 and F_8 generation of the Hamilton County ECB colony, as described in Dow's first report (MRID# 466958-01, submitted 11/05). The second report (MRID# 470112-01, submitted 12/06) indicated that the F_{11} generation was also tested, although no data or results could be found in either submission for this generation. Testing was done using artificial diet and a diagnostic concentration of Cry1F, similar to the procedure used for the routine annual resistance monitoring of Bt corn. Subsequent bioassays were also conducted on F_{13} and F_{18} generations using higher diagnostic concentrations of Cry1F.

A diagnostic concentration of 60 ng Cry1F/cm^2 was used for the F_7 and F_8 bioassays. This concentration is the same as that used for the annual resistance monitoring assays and approximates the LC_{99} of Cry1F-suscpetible ECB. At this concentration, few F_7 (2.7%) or F_8 (0.9%) larvae were killed in the trial. The reported mortality was substantially lower than the 48.3% mortality that was observed with the F_3 generation in the original monitoring work. With such high survival, the study authors were unable to generate a dose-response curve to calculate LC_{50} or EC_{50} values. In an ensuing test, F_{13} larvae (previously unexposed to Cry1F since the F_3 generation) were exposed to a diagnostic concentration of 600 ng Cry1F/cm^2, a 10 times greater toxin level than in the standard Cry1F concentration used for monitoring. These larvae also demonstrated high tolerance to Cry1F, with only 4.1% mortality (compared with 3.9% mortality in an unexposed control group). Some weight reduction (9.1%) relative to the unexposed control larvae was observed, although the exposed survivors were used to further continue the colony. A

final test of the F_{18} generation (used as a control group for the reciprocal cross test described later in this review) was conducted at concentrations up to 12,000 ng Cry1F/cm^2 (200-fold greater than the standard diagnostic concentration). No detectable mortality or growth inhibition was observed at even the highest 12,000 ng Cry1F/cm^2 concentration.

Considering the extremely low mortality (< 5%) to the diagnostic concentrations observed in the generations (F_7 - F_{18}) after initial (F3) selection, Dow concluded that the Cry1F tolerance trait is heritable in the Hamilton County ECB colony. Overall, tested ECB exhibited a resistance to Cry1F levels that exceeded 2,000 to 6,000 times the LC_{50} for susceptible ECB.

BPPD Review

BPPD agrees with Dow that the Cry1F challenge tests on later generations of Hamilton County ECB confirm that the population exhibits a high degree of resistance. Tested ECB were virtually immune to even the extremely large concentrations of Cry1F used in the bioassays (over 2,000 times greater than the ECB LC_{50} for Cry1F). Further, the trait is clearly heritable, as demonstrated by the apparently complete survival of F_{18} larvae (15 generations after initial selection) to highest test concentration.

The study report indicated that testing was also conducted on the F_{11} generation, although no description of the test, methods, or results could be located. This may have been an error, perhaps in reference to the F_{13} generation which was tested. It is also noted that the slide presentation (MRID# 466958-01) submitted to describe initial testing conducted during 2005 (i.e. tests through the F_8 generation) did not include a description of the methods used in the assays. Rather, abbreviated results tables and figures were presented. BPPD recommends that future reports be properly formatted with full descriptions of the methods and results. Despite these deficiencies in the reports, the study results clearly show that the Hamilton County ECB colony collected in 2004 is highly resistant to Cry1F in laboratory bioassays.

2) On Plant Survival

In addition to determining the heritability of the Cry1F tolerance trait, Dow also investigated the ability of the Hamilton County (HC) ECB colony to survive on Herculex Bt corn plants. Pest populations showing tolerance to Bt toxins are generally not considered "resistant" unless they are able to develop into reproducing adults on Bt expressing plants. Without the ability to survive on Bt plants, tolerant populations will not be able to reproduce and will not proliferate in the field.

Prior to conducting the whole plant assays, Dow challenged the HC population on leaf disks taken from Herculex (Cry1F) and non-expressing isoline plants. These assays were conducted in a greenhouse with an unselected lab colony serving as the control group. The results showed that 79.2% of the HC ECB survived after four days exposure to the Cry1F leaf disks (survival on the non-Bt leaf disks was 90.6%). Larval weights of the HC colony averaged 0.42 mg for those on Cry1F disks and 0.71 mg for the non-Bt disks. By comparison, the unselected control colony had no survival on the Cry1F disks and 97.8% on the non-Bt leaf disks. Although survival and

larval weights of the HC ECB were lower on the Cry1F disks relative to the control, this experiment demonstrated that the population may be able to survive and develop on Cry1F plants.

Whole plant assays were also conducted in greenhouse settings and were done with different generations (F_7-F_8 and F_{11}) from the HC ECB colony. In the first set of assays (detailed in MRID# 466958-01), F_7 and F_8 generation larvae were infested (30-40 per plant) on whorl and ear stage corn plants (Cry1F and isoline). An unselected laboratory colony was used as a control group and survival and larval weight were recorded 14-20 days after infestation. With whorl stage corn, the HC ECB (F_7) were found in approximately equal numbers on both Cry1F and isoline corn plants, although the overall number of larvae were recovered was small (<0.5 per plant). In contrast, high numbers of larvae from the control colony were recovered on isoline corn (>3 per plant), but only one larvae was found on Cry1F corn. HC larvae collected on Cry1F plants weighed less on average than those from isoline plants (~ 30 mg Cry1F vs. ~ 50 mg isoline), though it is unclear if the results are statistically significant. For reproductive stage corn (i.e. ear stage), similar numbers of larvae from the HC colony (F_8) were recovered on both Cry1F and isoline corn, although unlike the whorl stage results high numbers were recovered for both treatments (~ 7 per plant). Similar to the whorl stage corn test, the recovered HC larvae from Cry1F ear stage corn weighed less than those collected from the isoline corn (~ 40 mg Cry1F vs ~ 60 mg isoline; statistical analysis not presented). As could be expected, high survival was noted for the control colony (~ 9 per plant) on isoline corn while few larvae (<1 per plant) were found on the Cry1F corn plants. The study authors noted that the experiment was "inconclusive" because the surviving larvae were not allowed to fully develop into pupae and adults.

A second whole plant test was performed on the F_{11} and F_{12} generations from the HC colony. As with the first test, whorl and ear stage Cry1F corn plants were infested with 30 neonates per plant from the HC colony or an unselected control group. After three weeks, the plants were assessed for damage and living larvae were collected (from diapause) and for ear stage corn ultimately reared to adults. The results of this experiment showed that on whorl stage corn, few HC F_{11} larvae were recovered from either Cry1F or isoline corn. A total of four HC larvae were recovered from isoline corn and only one larva from Cry1F plants. This compares with 1.4 larvae/plant from the control colony on isoline corn (no control larvae were recovered on Cry1F plants). Cry1F corn plants showed some damage from HC feeding (2.3 avg. Guthrie score) though not to the extent of the isoline plants (4.5). In comparison, the unselected control group had Guthrie ratings of 5.3 on isoline corn and 1.0 (no visible feeding) on Cry1F plants. For ear stage corn, equal numbers of F_{12} HC larvae (68 total) were recovered on Cry1F and isoline corn, while only three larvae from the control colony were found on Cry1F corn (114 were found on isoline corn). Of the 68 HC larvae found on Cry1F corn, 45 completed diapause, 36 pupated, and 26 emerged as adults (38% of the total recovered larvae). Fewer HC larvae completed development on isoline corn (38% diapause, 31% pupae, and 28% eclosion) than on the Cry1F hybrids. Survival on ear stage corn is shown in table 1 below.

Table 1. Survival of larvae from the Hamilton County colony (HC) (F_{12}) and the unselected control colony (CC) on reproductive (ear) stage plants (reproduced from Table 2 in MRID# 470112-01).

Colony	Plant	Total Larvae Recovered	Larvae Completing Diapause	Pupae Recovered	Adult Eclosion
HC	TC1507 (Cry1F)	68	45 (66%)	36 (53%)	26 (38%)
HC	Isoline	68	25 (38%)	21 (31%)	19 (28%)
CC	TC1507 (Cry1F)	3	1 (33%)	0	0
CC	Isoline	114	68 (58%)	58 (51%)	48 (42%)

Based on the results from the greenhouse studies, Dow surmised that the Cry1F tolerance trait in the HC population allows larvae to grow and complete development on Herculex Cry1F corn plants. Although, it was noted that there are differences between greenhouse and field environments, the company concluded that the ability of HC larvae to survive Cry1F in the field is a "strong possibility."

BPPD Review

BPPD agrees with Dow that the greenhouse studies provide strong evidence that the Hamilton County ECB colony is capable of not only surviving on Cry1F plants, but fully completing development to adult moths. Survival and development was more pronounced on ear stage corn as opposed to whorl stage corn (few HC larvae were recovered from whorl stage Cry1F corn). It is unclear why this was observed and if there are any differences in toxin expression between the whorl and ear Cry1F corn plants used in the experiment.

Development to the adult stage is perhaps one of the greatest hurdles to the establishment and proliferation of resistance; many insects that are able to tolerate doses of Bt toxins in the laboratory are subsequently shown to be unable to complete development on Bt expressing plants. It is thought that fitness costs associated with the development of Bt tolerance traits often preclude survival on Bt plants. In fact, fitness costs may have played some roll in the case of the HC colony -- HC survival and development on Cry1F was somewhat less than the control colony on isoline corn. However, a significant number of HC larvae (38% of those recovered) were able to complete development on Cry1F corn to adults. It should be noted that the fecundity of the adults developing on Cry1F corn was not reported and it is not known whether these insects will be as reproductively successful as ECB developing on non-Bt corn. BPPD recommends that Dow continue to investigate any fitness costs (including development and fecundity) that might impact field survival and proliferation of Cry1F resistant ECB.

The greenhouse test results are perhaps the clearest indication that the HC colony collected in 2004 is in fact a Cry1F-resistant population. Tolerance and survival to adulthood on Cry1F plants could quickly lead to a field-resistant population capable of causing unexpected damage to corn. While BPPD notes that subsequent sampling in Hamilton County during 2005 did not produce similarly Cry1F-resistant ECB, it is highly recommended that Dow (via ABSTC)

continue to monitor the Hamilton County area in future growing seasons. Resistance monitoring data from the 2006 growing season has not yet been reviewed by BPPD.

3) Genetics of Resistance

As part of Dow's investigation of the Hamilton County ECB colony, studies were conducted to determine the genetic structure of the Cry1F resistance trait. Diet bioassays and crosses with known susceptible (laboratory) ECB colonies were utilized to evaluate dominance/recessiveness of the trait and potential sex linkage.

Crosses were conducted with HC (F_{13} and F_{18} generations) and control (Cry1F-susceptible) ECB colonies to produce F_1 larvae for the bioassays. Four groups were established: 1) HC; 2) HC male x control female; 3) HC female x control male; and 4) control. The F_{13} generation HC was crossed with a laboratory control colony while the F_{18} were bred with a field-collected control colony. Progeny obtained from the crosses were then exposed to a range of Cry1F concentrations in diet bioassays (up to a maximum of 12,000 ng/cm^2).

Both sets of crosses resulted in similar responses to Cry1F: HC ECB crossed with the susceptible colonies (i.e. groups # 2 and 3) remained sensitive to the toxin while the uncrossed HC group (i.e. group #1) was highly tolerant to Cry1F. The dose response curves of the two crosses (HC ♂ x CC ♀ and HC ♀ x CC ♂) were similar to that of the uncrossed control colony, although the crosses showed some increased ability to tolerate Cry1F relative to the control group. An EC_{50} or LC_{50} for the uncrossed HC groups could not be calculated because no mortality or growth inhibition was observed at the highest test concentration (12,000 ng/cm^2). The bioassay results from both sets of crosses are detailed in table 2 below.

Table 2. Results from reciprocal crosses of the Hamilton County (HC) colony with susceptible control colonies (CC or FC) (reproduced from information presented in Tables 3 and 4 in MRID# 470112-01)

Colony	N	EC_{50} (95% CI)	LC_{50} (95% CI)
F_{13} generation (CC = laboratory control colony)			
CC	383	1.74 (0.63 - 4.57)	9.28 (3.34 - 22.1)
HC	??	No mortality observed at the maximum test concentration	
HC ♀ x CC ♂	253	1.51 (1.05 - 2.18)	14.8 (9.07 - 24.5)
HC ♂ x CC ♀	382	6.37 (4.61 - 8.99)	24.9 (12.7 - 74.08)
F_{18} generation (FC = field control colony)			
FC	761	2.56 (1.96 - 3.33)	20.9 (15.8 - 26.7)
HC	127	No mortality observed at the maximum test concentration	
HC ♀ x FC ♂	713	1.54 (0.94 - 2.39)	25.1 (14.0 - 42.3)
HC ♂ x FC ♀	716	3.46 (2.35 - 5.07)	51.5 (12.7 - 74.1)

Given the results from the reciprocal crosses, Dow concluded that the Cry1F tolerance trait in the HC colony is recessive. This conclusion is supported by the crosses, each of which received a susceptible allele from the control colony; these (heterozygote) larvae remained almost as susceptible to Cry1F as the control groups and would be unlikely to survive in the field. The resistance is likely to be recessive because a heterozygote with a dominant resistance allele

would be expected to show more tolerance to Cry1F. Gender apparently played little role the crosses, as both the male and female combinations performed similarly in the bioassays. Therefore, it is likely that inheritance of the trait is autosomal and not sex-linked.

BPPD Review

The reciprocal crosses created between the HC colony and susceptible control colonies demonstrate that the Cry1F trait is likely to be a recessive, non-sex linked, single gene. As Dow has indicated in their report, heterozygotes containing one copy of the trait remain susceptible to Cry1F and will be unlikely to survive exposure to high dose Cry1F-expressing corn in the field. Rather, survival on Cry1F corn (as was demonstrated in the greenhouse trials) will be limited to homozygous individuals with two copies of the resistance allele.

Since the resistance trait is recessive, it may be difficult to detect in the field. Heterozygous larvae will not likely be found on Cry1F corn and ECB collected for resistance monitoring may not survive diagnostic test concentrations with only one copy of the resistance allele. It remains unclear how the resistance trait initially arose and was detected (the colony was established from a single egg mass collected from Hamilton County in 2004). One possibility is that the egg mass was homozygous for the resistance trait, presumably a very rare occurrence that happened to be detected by the annual ECB sampling. Another scenario is that the collected eggs were heterozygous and that the mating of the adults from this colony produced homozygote individuals capable of surviving the Cry1F diagnostic concentration.

4) Frequency of Resistance in Field Populations

The final (and ongoing) step in Dow's follow-up investigations of the Cry1F tolerant ECB from Hamilton County was an estimation of the prevalence of the trait in field populations from the county. To accomplish this objective, Dow (via ABSTC) conducted ECB sampling of the Hamilton County (HC) area during the 2005 growing season as part of the annual resistance monitoring work.

Three populations were collected directly from HC (543 total ECB) and their F_1 progeny were screened against a Cry1F diagnostic concentration. The test concentration, 60 ng/cm^2, has been used for the annual Cry1F corn monitoring (including for the 2004 Cry1F-tolerant HC colony) and is known to produce 100% mortality to susceptible ECB. None of the tested HC progeny survived the diagnostic Cry1F concentration, indicating that the Cry1F tolerance trait was below the level of detection.

Dow conducted an analysis of the potential resistance allele frequency based on the 2005 sampling and bioassay results. The company reasoned that of the collected ECB (which included mixed sex samples and assuming a 1:1 sex ratio), 330 mating were likely to have occurred with none of the progeny surviving the diagnostic concentration. Using Hardy-Weinberg genetics (see Appendix B in MRID# 470112-01), Dow calculated that based on 330 matings, the resistance allele frequency was less than 0.028 in the field. Further calculations were conducted (based on the probability of not detecting the resistance allele) to determine that

there was an 80% probability the resistance allele frequency was below 0.036 and a 95% probability it was below 0.049. These resistance allele frequencies are also below the level of concern (as detailed in the ABSTC monitoring plan -- see review in BPPD 2004a), which for recessive resistance is a resistance allele frequency of 0.075. Dow determined that if the resistance allele frequency was 0.075 than the probability of detecting the trait in 330 matings would be greater than 99.9%.

In addition to the work with HC, Dow noted that ECB sampling in neighboring counties remained susceptible to Cry1F and that no reports of unexpected pest damage were received in HC or elsewhere. The company has also indicated that HC will be included in the 2006 sampling and monitoring program.

BPPD Review

BPPD notes that the 2005 Cry1F monitoring data including results for Hamilton County collections were submitted and reviewed separately (see BPPD 2007). These results showed that the Cry1F resistance trait could not be detected from the sampled ECB. However, BPPD is still concerned about the Hamilton County area and the potential for field scale resistance to develop.

Dow's probability analysis of their 2005 collections showed that is extremely unlikely that the resistance allele occurred at a frequency above the level of concern. While this conclusion is valid for the populations collected, there remains the possibility that the resistance allele may occur in areas that were not sampled. Field resistance will likely occur initially on a local scale (conceivably a single field) and such localized resistance may evade detection given the limited sampling capability of the resistance monitoring program. Therefore, BPPD strongly recommends continued extensive resistance monitoring in Hamilton County for the 2007 growing season and beyond (Dow has stated that HC was to be included in the 2006 program, which has not yet been reviewed by BPPD). Since ECB are known to be mobile, it is also recommended that surrounding counties be included with the monitoring. Should these efforts detect that the Cry1F resistance allele is proliferating, an appropriate remedial action plan specific to Hamilton County should be put into place.

Overall Conclusions

Based on the initial Hamilton County (HC) data and the follow-up testing reviewed here, BPPD can conclude that the 2004 HC colony is resistant to the Cry1F toxin expressed in Herculex corn. The colony meets all of the major criteria for resistance: the trait is heritable (determined to be a single recessive gene), the trait confers survival to high levels of Cry1F, and ECB with the trait are capable of developing to adults on Cry1F-expressing corn plants. BPPD notes that this is the first documented case of pest resistance to a high dose Bt crop; though it should also be noted that no documented cases of field failure or resistance have been detected in HC or elsewhere. Given that the sampling strategy for the annual resistance monitoring program covers only a small portion of corn-growing regions, it is possible that such traits are more common than previous thought. Other cases may remain undetected if significant field-level effects are not observed.

Dow contends that because the trait was not detected in 2005, the resistance allele frequency would is below the level of concern and a remedial action plan is not needed. While BPPD acknowledges this conclusion from the 2005 monitoring data, the lack of detection does not ensure that the resistance allele does not exist (the 2004 results show that the allele does in fact exist in nature). Rather, improved sampling or more sensitive detection methods may be warranted. BPPD believes that additional data (i.e. monitoring in subsequent growing seasons) are needed to verify Dow's conclusion and decision not to implement a remedial action plan. As described in section 4 above, resistance development to Bt corn will likely be localized and not easily detected by limited sampling. Therefore, continued resistance monitoring of the Hamilton County area (including surrounding counties) is essential to maintaining the IRM program for Cry1F corn. Reports of unexpected pest damage should be noted and promptly investigated since the HC population was shown to be tolerant of whole corn plants. A remedial action plan may need to be considered if the resistance trait is detected in subsequent growing seasons.

BPPD recommends that Dow (or ABSTC) continue to maintain and investigate the HC colony to characterize the Cry1F resistance. This colony may yield valuable information on Cry1F resistance that may aid the understanding and improvement of IRM plans. Additional monitoring techniques (such as the F_2 screen) should also be considered to determine the frequency of recessive resistance. Further, it is recommended that Dow investigate cross-resistance potential between Cry1F and Cry1Ab since the Hamilton County ECB population collected in 2004 exhibited tolerance to both toxins.

References

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13544

R151204

Chemical: Bacillus thuringiensis Cry 1F protein and the genetic material necessary for its production (plasmid insert PHI 8999)in corn

PC Code: 006481
HED File Code: 41400 BPPD IRM
Memo Date: 8/7/2007
File ID: DPD336805
Accession #: 000-00-9003

HED Records Reference Center
9/21/2007